

Universidade de Lisboa  
Faculdade de Ciências  
Departamento de Biologia Animal



**Factors affecting southern water vole (*Arvicola sapidus*) detection and  
occupancy probabilities in Mediterranean farmland**

**Dinora Sofia Jorge Peralta**

Dissertação  
Mestrado em Ecologia e Gestão Ambiental

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Orientadores:  
Professora Doutora Maria de luz Mathias  
Doutor Ricardo Pita

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## **Nota Prévia**

Na escrita desta tese optou-se pela língua inglesa por ser a língua universalmente aceite nas publicações científicas internacionais. A tese foi organizada em 3 capítulos, um de contextualização geral do problema, um capítulo principal em forma de artigo científico, e um capítulo de síntese dos principais resultados encontrados. Na escrita do capítulo principal, a opção pelo formato de artigo científico prendeu-se com o facto de este ser o formato mais comum na disseminação de estudos científicos, e por no futuro se pretender submeter o trabalho a uma revista na área da ecologia e/ou zoologia indexada na base *ISI*. Assim, embora o artigo não tenha seguido a formatação específica de nenhuma revista em particular, a estrutura corresponde ao que é normalmente aceite em publicações científicas (e.g. Elsevier, ou Springer). A autora esclarece que os trabalhos foram feitos em colaboração, e que em todos eles participou ativamente na sua recolha, análise, e redação.

## Resumo

A agricultura tem uma longa história, desde as suas origens no leste Mediterrânico há 10 000 anos atrás, até a uma das formas mais generalizada dos atuais usos do solo na Europa. Geralmente, as paisagens agrícolas apresentam-se sob a forma de mosaicos com diferentes usos do solo, dominadas por culturas e pastagens nativas ou melhoradas utilizadas para pastagem. Além de espacialmente heterogêneos, os mosaicos agrícolas também são dinâmicos em diversas escalas temporais, sendo que, espécies que vivem em áreas agrícolas podem também interagir com as mudanças ambientais ao longo do tempo. Na Europa, as paisagens agrícolas desempenham um papel extremamente importante para a conservação da biodiversidade, visto que muitas espécies dependem diretamente dos sistemas agrícolas tradicionais. Contudo, a diversidade destas paisagens tradicionais está a decrescer drasticamente, quer devido ao abandono das terras, quer devido à intensificação do uso dos solos. Em particular, a intensificação da agricultura é comumente apontada como uma ameaça à sobrevivência de muitas espécies, normalmente adaptadas aos sistemas agrícolas tradicionais. Desde modo, compreender de que forma é que a intensificação da agricultura afeta a biodiversidade tornou-se num dos objetivos principais quer da ecologia, quer da conservação da vida selvagem. No entanto, alcançar este objetivo traz também muitos desafios, tanto conceptualmente, como metodologicamente, sendo que neste contexto, o conceito de metapopulação será particularmente útil na compreensão de como a perda e a fragmentação dos habitats pode afetar a persistência de espécies espacialmente estruturadas.

Atualmente, o conceito geral de metapopulação consiste na ideia de que habitats de menores dimensões e mais isolados apresentam menor probabilidade de serem ocupados, enquanto que habitats maiores e menos isolados estão mais propensos a serem colonizados. Em condições de equilíbrio, a persistência de uma metapopulação a uma escala regional, resulta do equilíbrio entre eventos de extinção e colonização local. Assim, os modelos metapopulacionais podem ser particularmente úteis para avaliar de que forma é que a transformação da paisagem pode afetar a dinâmica extinção/colonização e a persistência a longo prazo das populações, desde que (i) fragmentos de habitats adequados possam ser diferenciados a partir da matriz envolvente; (ii) todas as populações locais apresentem determinado risco de extinção, em algum momento; e (iii) a dispersão entre habitats e os eventos de colonização locais ocorram lentamente, de modo a que a dinâmica das populações dentro dos habitats seja assíncrona. Normalmente, o foco dos modelos de metapopulações incide sobre a dinâmica de ocupação das

espécies nos habitats, enquanto a dinâmica das populações locais é muitas vezes negligenciada. No entanto, quando se trabalha com ocupação de espécies, é fundamental ter em consideração a possibilidade de que uma espécie pode estar presente num habitat e não ser detetada durante a amostragem. Deste modo, se a deteção imperfeita não for contabilizada nos modelos, estas "falsas ausências" podem levar a inferências incorretas sobre a dinâmica local da espécie alvo. Para solucionar este problema, foram desenvolvidos modelos que incorporam explicitamente deteções imperfeitas no processo de modelagem para permitir uma estimativa imparcial da probabilidade de ocupação (dada como a fração de sítios ocupados), e das co variáveis que a afetam.

Em modelos de ocupação que têm em conta deteções imperfeitas, a probabilidade de deteção é normalmente assumida como sendo menor do que 1. Para incorporar deteções imperfeitas, é necessário que, pelo menos, alguns locais sejam amostrados mais do que uma vez, dentro de um período de tempo relativamente curto, envolvendo, portanto, tanto uma replicação espacial como temporal. A ideia básica associada a estes modelos é que dentro do período de tempo da amostragem, assume-se que não ocorrem mudanças na ocupação desses mesmos habitats (ou seja, os habitats ou estão sempre ocupados ou desocupados pelas espécies), sendo que as mudanças só podem ocorrer entre diferentes períodos de amostragem, devido a eventos de colonização e extinção local. Assim, com base em amostragens repetidas de presença/ausência das espécies, estes modelos ajudam a estimar a probabilidade de deteção de pelo menos um indivíduo da espécie-alvo durante um período de amostragem, sabendo que os indivíduos estão presentes na área de estudo. Contudo, apesar de serem simples de planejar, os métodos de amostragem necessários para desenvolver estes modelos são muitas vezes difíceis de implementar, na medida em que é necessário uma replicação dos locais amostrados, o que muitas vezes requer um compromisso entre o número de pontos de amostragem distribuídos em toda a área de interesse/inferência (replicação espacial), e o número de replicas de amostragem em cada local (replicação temporal). Para compensar este potencial problema, uma abordagem comum é a realização de várias amostragens independentes numa única visita, usando um ou múltiplos observadores, ou então proceder-se à amostragem de várias parcelas inseridas num local maior, numa única visita (replicação espacial).

O rato-de-água (*Arvicola sapidus* Miller 1908) é um pequeno mamífero restrito à Península Ibérica e França, sendo classificado como "Vulnerável" pela União Internacional para a Conservação da Natureza (IUCN). As populações desta

espécie encontram-se em declínio em vários locais da sua distribuição, principalmente devido à perda e fragmentação de habitats resultantes das atividades humanas. Tipicamente, os habitats adequados ao rato-de-água estão associados às margens estáveis de cursos de água, com solo lamacento dominado por herbáceas ou vegetação arbustiva. À semelhança do seu congénere *A. amphibious*, o rato-de-água normalmente forma colónias discretas, facilmente reconhecíveis, sendo muitas vezes vistas como subunidades distintas de uma população maior ou metapopulação. Assim, o rato-de-Água apresenta-se como uma espécie ideal para uma abordagem metapopulacional, a qual poderá gerar informações úteis relativas à avaliação da dinâmica (meta)populacional desta espécie. Geralmente, o rato-de-Água forma pequenos trilhos onde os indivíduos costumam circular e onde produzem latrinas com típicos dejetos cilíndricos, muitas vezes usadas como indícios indiretos da presença da espécie. Desta forma, a presença destas latrinas é geralmente utilizada para realizar amostragens em grande escala, no sentido de se inferir acerca do estado das populações de rato-de-Água, incluindo estudos que avaliam os fatores que afetam os seus padrões de ocupação em diferentes sistemas ecológicos, como zonas montanhosas, zonas agrícolas, ou sistemas de lagoas temporárias. No entanto, nenhum destes estudos teve em conta as deteções imperfeitas, o que pode limitar a força das inferências sobre as variações de ocupação, e, assim, a eficácia dos esforços de conservação recomendadas para a espécie.

Dada a necessidade de avaliar as tendências das populações de ratos-de-Água, bem como a falta de estudos de modelação das probabilidades de ocupação da espécie, tendo em conta deteções imperfeitas, este estudo tem como objetivo facultar mais pistas sobre os fatores que afetam os padrões e dinâmica de ocupação desta espécie em paisagens agrícolas Mediterrânicas, testando quais as co-variáveis ambientais e relacionados com as amostragens (esforço de amostragem, experiência do observador, chuva) podem eventualmente produzir variações na probabilidade de deteção da espécie. Especificamente, os habitats adequados para a espécie foram identificados e mapeados dentro de uma área de cerca de 247,6 ha, e em seguida foi amostrada a presença da espécie em cada habitat. Esta amostragem foi feita com base em típicos da presença da espécie, usando dois observadores, e empregando diferentes esforços de amostragem. Este procedimento foi repetido em duas sessões de amostragem, que correspondem ao início da época de reprodução (novembro-dezembro) e à época em que as populações estão presumivelmente perto de seu pico de abundância (fevereiro-março). Em seguida, foram desenvolvidos modelos de ocupação espacialmente

explícitos para estimar as probabilidades de detecção e ocupação sazonais dos ratos-de-Água em relação às características dos habitats.

De acordo com as previsões da teoria da metapopulação, os resultados obtidos mostram que a ocupação dos habitats pelos ratos-de-água está positivamente relacionada com a área do habitat e negativamente relacionada com o isolamento dos habitats. Além disso, e de acordo com as preferências da espécie em relação ao habitat, a presença de água foi significativa para a probabilidade de ocupação, contudo, as variáveis testadas para a vegetação (% coberto vegetal e altura do coberto) não mostraram suporte no ranking dos modelos. Por outro lado, esperava-se que a probabilidade de detecção variasse, não apenas com as características da vegetação presente nos habitats, mas também com a experiência do observador na condução das amostragens aos sinais típicos da presença da espécie, com o esforço de amostragem empregue, com o tempo decorrido desde o início do estudo, e com as condições meteorológicas durante as amostragens. Contudo, nenhuma destas variáveis mostrou qualquer suporte aquando do ranking dos vários modelos testados. No geral, espera-se que os resultados obtidos proporcionem uma melhor compreensão no que diz respeito às respostas das populações de ratos-de-água aos atuais usos do solo em paisagens Mediterrânicas fragmentadas. É ainda importante discutir os possíveis efeitos de probabilidades de detecção heterogêneas na força das inferências que podem ser feitas, bem como as suas implicações em programas de monitorização que visem a conservação da espécie a grandes escalas (de paisagem e nível regional)

**Palavras-chave:** Agricultura; Metapopulação; Modelos de ocupação; Detecção imperfeita; Indícios de presença



## Abstract

In Europe, agricultural landscapes may play an extremely important role to biodiversity conservation, as many species depend directly on traditional farming systems. However, the diversity of traditional agricultural landscapes is decreasing dramatically, either due to land abandonment or crop production intensification. Understanding the effects of agricultural intensification on farmland biodiversity has thus become a main goal in both wildlife ecology and conservation. In this context, the metapopulation concept has provided a useful framework to understand how habitat loss and fragmentation may affect the persistence of species that is spatially structured. The southern water vole (*Arvicola sapidus*) usually forms discrete and easily recognizable breeding colonies, which are often seen as subunits of a larger patchy population or metapopulation, and thus is an ideal species to examine whether a metapopulation approach can yield useful insights for assessing its population dynamics. In this study we addressed this issue, analyzing the factors shaping the occupancy patterns and dynamics of this species in Mediterranean farmland, based on presence-sign searches in two seasons, and taking into account for imperfect detection. A spatially explicit modeling approach was used to test the following predictions: detectability of southern water vole presence signs should be affected by the sampling effort employed during the surveys, observer experience, local vegetation characteristics, and weather conditions; while occupancy should be mostly affected by patch size, isolation, presence of water, and vegetation variables. Results indicated that detectability was relatively high ( $\approx 71\text{--}81\%$ ), though contrary to the initial predictions, there was no support for none of the effects tested. Occupancy probabilities were relatively low ( $\approx 29\text{--}31\%$ ), and according to predictions from metapopulation theory and the species habitat preferences, was positively related to the patch area and presence of water, and negatively related to patch isolation, while vegetation variables tested showed no significant effects. Overall, this study provided important insights regarding the responses of southern water vole patchy populations to current land-uses in fragmented Mediterranean farmland; and the possible effects of imperfect detection on the strength of inferences made, and its implications for large-scale (landscape- and regional-level) monitoring programs targeting the conservation of the species.

**Keywords:** Agriculture; Metapopulation; Occupancy models; Imperfect detection; Presence signs

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# **1. General Introduction**

## **1.1 Landscape and biological diversity in agricultural areas**

Agriculture has a long history, from its origins in the eastern Mediterranean 10 000 years ago, to the most widespread forms of current land uses in Europe. In general, agricultural landscapes may show a wide range of ecological conditions and may differ considerably in terms of their biodiversity, depending on a combination of factors, such as soil condition, water availability, climate, slope, and management options at multiple spatial and temporal scales (Henle et al., 2008). Typically, agricultural landscapes are mosaics with different land-uses dominated by crops, and improved or native grasslands used for pasture. Wood cover usually occurs in woodlots and strips of planted trees, while natural or semi-natural vegetation is largely restricted to small habitat patches along streams, roads and field boundaries (Luck and Daily, 2003; Bennett et al., 2006). These land mosaics may function as a cluster of habitats for plant and animal species, with some species occurring only in the natural or semi-natural elements of land mosaics, while others readily use the managed land, including crops, tree plantations or urban habitats (Bennett et al., 2006). Besides spatially heterogeneous, agricultural land mosaics are also dynamic at multiple temporal scales, and thus species living in farmland areas may also interact with environmental change across time (Tscharntke et al., 2005; Lindenmayer and Fischer, 2006; Bennett et al., 2006; Bennett and Saunders, 2010).

In Europe, agricultural landscapes may play an extremely important role to biodiversity conservation (Bennett et al., 2006), as many species depend directly on traditional farming systems (Henle et al., 2008; Stoate et al., 2009). Indeed, it is estimated that 50% of all species in Europe depend on agricultural habitats, including endangered and endemic species (Stoate et al., 2009). However, the diversity of traditional agricultural landscapes is decreasing dramatically, either due to land abandonment or crop production intensification (Stoate et al., 2001; Henle et al., 2008). In particular, agricultural intensification is commonly referred to threaten the survival of many species otherwise adapted to traditional agricultural systems (Stoate et al., 2001; Henle et al., 2008; Stoate et al., 2009). In the past decades, the remarkable increase in intensive agriculture has significantly altered farmland landscapes, with detrimental effects on farmland biodiversity (Stoate et al., 2001; Tscharntke et al., 2005; Lindenmayer and Fischer, 2006; Henle et al., 2008). Main changes associated agricultural intensification include the incremental loss and fragmentation of natural vegetation, simplification and degradation of habitats, spread of exotic species, increases in the use of chemical pesticides and

fertilizers, degradation of soil and water systems, and expansion of human settlements and road systems (Stoate et al., 2001; Tscharntke et al., 2005; Bennett et al., 2006).

From the conservation point of view, the rapid changes in both structural and functional characteristics of many farmland mosaics have raised increased concern, particularly regarding their effects on the long-term capacity of the land mosaic to support native biodiversity, keeping the ecological processes (Bennett et al., 2006). Understanding the effects of agricultural intensification on farmland biodiversity has thus become a main goal in both wildlife ecology and conservation (Stoate et al., 2001; Tscharntke et al., 2005; Bennett et al., 2006; Henle et al., 2008). However achieving this main goal also poses many challenges both conceptually and methodologically. In particular, identifying the effects of land-use change on species that are restricted to specific habitat patches and have limited dispersal abilities has been a major focus for ecologists and conservation managers (Harrison, 1991; Driscoll, 2007). In this context the metapopulation concept has provided a useful framework to understand how habitat loss and fragmentation may affect the persistence of species that spatially structured, either as patchy populations or classic metapopulations (Driscoll, 2007; Pita et al., 2013).

## **1.2 The metapopulation concept and its application in biodiversity conservation**

Habitat loss and fragmentation typically result in a decrease in local habitat patch sizes, and an increased isolation among patches, thus increasing local extinction probabilities and preventing colonization events by impairing animal movements across the landscape (Lindenmayer and Fischer, 2006). Such subdivision of natural populations into subpopulations or local populations that are linked by dispersal movements lies at the core of the metapopulation concept (Hanski, 1998; Lindenmayer and Fischer, 2006; Bennett and Saunders, 2010; Frey et al., 2012;). The general idea behind current metapopulation thinking is that smaller and more isolated patches are more likely to become extinct, while larger and less isolated patches are more likely to be colonized (Lindenmayer and Fischer, 2006; Bennett and Saunders, 2010). Thus, larger patches will be occupied more often and over longer periods than smaller patches, whereas isolated patches will remain empty for longer periods than patches close together (Opdam, 1991). Under stable equilibrium, metapopulation persistence at the landscape and regional scales results from the balance between local extinction and colonization events.

Metapopulation models may thus be particularly useful to assess how landscape transformation may affect extinction-colonization dynamics and long term persistence, as long as (i) suitable habitat patches can be differentiated from the surrounding unsuitable matrix; (ii) all local populations have some risk of extinction at some point; and (iii) interpatch dispersal and local colonization events occur at low rates, such that local population dynamics within patches are asynchronous (Lindenmayer and Fischer, 2006; Schooley and Branch, 2007).

In the simplest, spatially-implicit models or classical models as conceived originally by Levins, all patches are considered identical (Vandermeer and Carvajal, 2001; Lindenmayer and Fischer, 2006; Bennett and Saunders, 2010). Alternative metapopulation models, referred to as mainland-island metapopulation model, state that one subpopulation is significantly larger and more permanent than all the others, and serves as primary source population for smaller subpopulations (Lindenmayer and Fischer, 2006; Bennett and Saunders, 2010). Other types of metapopulation models include the patchy model, in which migration among habitat patches is so frequent that the patches function as a single demographic unit (Driscoll, 2007), and the nonequilibrium model, in which movement among subpopulations is so limited that each one functions as a separate population (Driscoll, 2007). Recently, efforts in modeling metapopulations rely mostly on spatially-explicit models, which assume that patches may vary in characteristics such as their size and isolation to the nearest habitat patches (Hanski, 1998; Bennett and Saunders, 2010; Frey et al., 2012). Further improvements in metapopulation modeling emerged with the development of spatially-realistic metapopulation models, which incorporate considerations on the spatial location and geometry of suitable patches, and other habitat attributes such as internal quality (Hanski and Ovaskainen, 2003). Most popular spatially-realistic metapopulation models include simulations and incidence function models, which are able to make quantitative predictions about metapopulation dynamics (Hanski and Ovaskainen, 2003; Driscoll, 2007).

Although the mathematical and conceptual analysis of metapopulations may differ in their assumptions and complexity, in each case the focus of metapopulation models is on the occupancy dynamics of habitat patches, while local population dynamics is often disregarded. This common feature to most metapopulation models make them particularly attractive to estimate patch occupancy dynamics, both because they are analytically tractable, and simply require presence-absence sampling schemes, which are relatively easy to plan and implement (Bailey et al., 2004; MacKenzie and Bailey, 2004). However, when working with occupancy, it is crucial to consider the possibility that a species may

be present on a site but not detected during the survey (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005). Although species presence-absence surveys are commonly used in metapopulation models, they can not be used to confirm that a species is absent from a site. Such 'false absences' lead to incorrect inferences if the imperfect detection of the species is not accounted for (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005). For instance occupancy probability may be underestimated, colonization and local extinction rates can be biased; and habitat relationships may as well be misleading, particularly if detectability also changes across habitat types or other source of variation (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005). To account for this problem, models explicitly incorporating imperfect detection into the modelling process have been developed to allow unbiased estimation of occupancy probability (given as the fraction of occupied sites), and the covariates affecting it (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005).

### **1.3 Occupancy models of metapopulation dynamics under imperfect detection**

A general assumption of occupancy models that take into account imperfect detection is that detection probabilities is in most cases less than 1 (MacKenzie et al., 2002; Bailey et al., 2004). A general requirement is that at least some locations are surveyed in more than one occasion within a relatively short period of time, thus involving both spatial and temporal replication (Bailey et al., 2004; Mackenzie and Royle, 2005; Bailey et al., 2007). The basic idea underlying these models is that within a given time period, patches are assumed to be closed to changes in occupancy (i.e., sites are either always occupied or unoccupied by the species), and changes may only occur between different time periods due to colonization and local extinction events (MacKenzie et al., 2002; MacKenzie et al., 2003; Mackenzie, 2005; Mackenzie and Royle, 2005; Bailey et al., 2007). Additional assumptions include: (i) detections occur independently at habitat patches; (ii) occupancy and detection probabilities do not vary across habitat patches and time, except when differences can be modeled with covariates; and (iii) the study species is identified correctly (Bailey et al., 2007).

In occupancy models under imperfect detection occupancy probability may be modeled as a function of site-specific covariates that do not change during the season, such as habitat type, size, quality, and isolation; while detection probability may be modeled as a function of either site-specific or survey-specific covariates, such as weather conditions, sampling technique and effort used, observer experience, local population density, and seasonal or behavioural patterns (Bailey et al., 2007). Based on repeated presence/absence (detection-no detection) surveys, these models estimate the probability of detecting at least one individual of the target species during a sampling occasion, given that individuals are present in the study area (MacKenzie et al., 2002; Bailey et al., 2004). Species occupancy relative to site covariates is then estimated while accounting for detection probabilities. Despite simple to plan, sampling designs required to develop these models are often hard to implement, because the implied replication at sampled sites needed to estimate detection probabilities, often creates a trade-off between the number of sample sites distributed across the area of interest/inference (spatial replication), and the number of repeated surveys at each site (temporal replication) (Bailey et al., 2004; Mackenzie and Royle, 2005; Bailey et al., 2007). To offset this potential problem a common approach is to conduct multiple independent surveys within a single visit, either by using single or multiple observers (MacKenzie et al., 2003; Mackenzie and Royle, 2005); or surveying multiple plots within a larger site on a single visit (spatial replication) (MacKenzie et al., 2003; Mackenzie and Royle, 2005).

Most simple occupancy models use multiple surveys within a single pre-defined period of time or season, short enough to guarantee the closure assumptions. Although single-season occupancy models provide an indication of the current patterns in occupancy within that season (i.e. a snapshot of the population at a single point of time), and may allow inferences on implicit dynamics over multiple seasons (Moilanen, 1999; MacKenzie et al., 2005; Pichancourt et al., 2006; Hossack et al., 2013; Ellis et al., 2014), properly understanding the process underlying those patterns may require the use of multi-season explicit dynamic occupancy models, which allow to estimate local colonization and extinction probabilities over time (MacKenzie et al., 2003; Mackenzie, 2005; Bailey et al., 2007). More complex models may include multi-species or (meta)community-level occupancy studies over multiple seasons, thus allowing to test predictions regarding species interactions also (Dorazio and Royle, 2005; Dorazio et al., 2010); or may incorporate stage structured (Sutherland et al., 2012) and local demography and dispersal explicitly (e.g. Sutherland et al. in press). Independently of the type of occupancy model used, it is worth noting that approaches assuming detection



probabilities less than 1, represent a substantial improvement to make inferences regarding metapopulation dynamics, over approaches that do not consider imperfect detection (MacKenzie et al., 2003; Mackenzie, 2005; Bailey et al., 2007). These models may be applied to real world conservation problems, such as prioritizing areas and prescribe land-use planning for many species of conservation concern.

#### **1.4 The southern water vole (*Arvicola sapidus*) in Mediterranean farmland as a study case**

The southern water vole (*Arvicola sapidus*, Miller 1908 Rodentia, Cricetidae) (Fig. 1) is a small mammal restricted to the Iberian Peninsula and France, being classified as 'Vulnerable' by the International Union for Conservation of Nature (IUCN) Red List (Rigaux et al., 2008). Populations of this species are declining in most parts of its range, mainly due to habitat loss and degradation resulting from human activities (Rigaux et al., 2008; Pita et al., 2010; Ventura, 2012; Mate et al., 2013). Suitable habitats for the southern water vole are mostly associated to stable banks of water courses, in muddy soil, dominated by herbaceous or shrub vegetation (Fedriani et al., 2002; Román, 2007; Román, 2010; Ventura, 2012; Mate et al., 2013). The species is largely dependent on grasses, sedges, and reeds, which are an important source of food, nesting site, and protection from predators (Román, 2007; Román, 2010; Ventura, 2012; Mate et al., 2013;) (Fig. 2). Similarly to its congeneric *A. amphibious*, the southern water vole usually forms discrete and easily recognizable breeding colonies, which are often seen as subunits of a larger patchy population or metapopulation (Fedriani et al., 2002; Centeno-Cuadros et al., 2011; Mate et al., 2013). These local populations are often separated by hundreds of meters from each other, which are often connected only through dispersal movements (mean of about 600-800m, Román, 2007, Centeno-Cuadros et al., 2011). The southern water vole is thus an ideal species to examine whether a metapopulation approach can yield useful insights for assessing its population dynamics (e.g. Fedriani et al., 2002; Pita et al., 2013).

Within habitat patches individuals typically present strong site fidelity to their home ranges, which in Mediterranean farmland averaged about 900 m<sup>2</sup> (Pita et al., 2010, 2013). Mean lifespan of wild ranging animals is around 3–5 months, and mating system seems to be both habitat- and density-dependent, occurring either within polygynous or monogamous systems (Román, 2007; Pita et al., 2010, 2011, 2013). Southern water voles show predominantly crepuscular activity during the

dry season with peaks at dawn and dusk, although they could also have some nightlife (Ventura, 2012). During the wet season they may present more diurnal activity and males are more active than females (Ventura, 2012). The animals build, maintain and repair the paths through which they usually circulate and that are essential to the colonies survival (Román, 2007; Román, 2010; Ventura, 2012). Within their pathways, individuals usually produce latrines with typical cylindrical droppings (Fig. 3), which are often use as indirect signs of the presence of the species, and have been used to conduct large-scale surveys of its populations, including studies aiming to assess the factors affecting its occupancy patterns across different ecological systems, including mountain areas (Mate et al., 2013), farmland areas (Román, 2003; Román, 2010), and pond systems (Fedriani et al., 2002). However, none of these studies incorporated imperfect detection, which may limit the strength of inferences regarding species occupancy variation, and thus the effectiveness of conservation efforts recommended for the species.



**Fig. 1** – Wild ranging southern water vole captured near Sines, south-west Portugal (photographed by R. Pita).





**Fig. 2** – Typical suitable habitat for the southern water vole in south west Portugal Mediterranean farmlands (photographed by R. Pita)



**Fig. 3** – Example of latrine with typical cylindrical droppings of southern water voles set on a runway made on grasses (photographed by R. Pita).

## **1.5. Main objectives and expected results of this study**

Given the need to assess population trends for the threatened southern water vole, and the lack of studies modeling occupancy probabilities of the species accounting for imperfect detection, this study aimed at providing further insights on the factors shaping the occupancy patterns and dynamics of this species in Mediterranean farmland, while taking into account for eventual variation in detection probabilities relative to environmental and survey-specific covariates. Specifically, we identified and mapped suitable habitats for the southern water vole within a Mediterranean farmland area of about 247.6 ha, and then surveyed the species at each patch, based on presence sign searches, using double observer approach, and employing different sampling efforts. We repeated surveys in two sampling seasons that corresponded to the beginning of the breeding season (November-December) and the time at which populations are presumably close to their peak abundance (February-March) (Román, 2007; Pita et al., 2013). We then used a spatially-explicit modelling approach with implicit dynamics (MacKenzie et al., 2005; Hossack et al., 2013; Ellis et al., 2014) to assess seasonal occupancy probabilities of southern water voles relative to patch area, isolation, vegetation composition and structure, and presence of water; while simultaneously testing for both environmental and survey-specific covariates (e.g. observers experience, sampling effort used, weather conditions) potentially affecting the detection probabilities of the species.

In line with the predictions from metapopulation theory, we expect that patch occupancy of southern water voles should be positively related to patch size, and negatively so with patch isolation (Hanski, 1998; Hanski and Hanski, 1999; Fedriani et al., 2002; Hanski and Ovaskainen, 2003; Pita et al., 2013). In addition, according to habitat preferences of the species (Román 2007; Pita et al. 2011), the probability of patch occupancy should increase with the presence of water, and the increasing of both vegetation cover and high. On the other hand we expect that detection probabilities should vary not only with vegetation characteristics within habitat patches, but also with the experience of the observer in conducting presence sign searches of the species, the sampling effort employed, the time elapsed since the beginning of the study, and the weather conditions during the surveys. In particular, detection probabilities should be higher for experienced observers, at increased sampling efforts, and throughout the time of the study, decreasing for instance with increasing rainfall, which may wipe off voles droppings (Román 2003; Pita et al., 2013).

Overall, results from the study are expected to provide further insights regarding the responses of southern water vole patchy populations to current land-uses in fragmented Mediterranean farmland; and to discuss the possible effects of heterogeneous detection probabilities on the strength of inferences made, and its implications for large-scale (landscape- and regional-level) monitoring programs targeting the conservation of the species.

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## **2. Paper: Factors affecting southern water vole (*Arvicola sapidus*) detection and occupancy probabilities in Mediterranean Farmland**

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### **Abstract**

Although species presence/absence surveys are commonly used in occupancy modelling of spatially structured populations, predicting species presence requires consideration of detection probability of individuals. Despite widely acknowledged, imperfect detection is still often disregarded in many studies aiming to estimate species occupancy patterns and dynamics. This is the case of the southern water vole (*Arvicola sapidus*), a threatened small mammal from southwest Europe that has experienced strong population declines mainly due to habitat loss and fragmentation. Here we modeled for the first time the occupancy probability of southern water vole patchy populations in Mediterranean farmland, while accounting for imperfect detection. Based on repeated presence sign searches conducted during two seasons (November-December and February-March), we used a spatially explicit occupancy modeling approach with implicit dynamics to test the effects of vegetation characteristics, sampling effort, observer experience, and rainfall on the detection probability of the species. We then assessed whether occupancy could be related to patch size, isolation, vegetation variables, and presence of water, after correcting for imperfect detection. Results indicated that detection probability of presence signs was relatively high ( $\approx 0.71$  and  $0.81$  for the

first a second season, respectively), and was little affected by all patch- and survey-specific covariates considered. After controlling for imperfect detection, seasonal estimates of occupancy were relatively low and constant ( $\approx 0.31$  and  $0.29$  for the first a second season, respectively), and according to predictions, were positively related to patch size and presence of water, and negatively so to patch isolation. Overall, our study provides the first estimates of detectability and occupancy for the southern water vole in Mediterranean farmland, while it also identified the local and survey-specific factors affecting these estimates. We suggest that designing monitoring programs for southern water vole patchy populations at landscape and regional scales, based on replicated presence sign surveys within limited time intervals, may be a reliable approach to properly estimate voles occupancy patterns and dynamics, as well as for reducing bias in patch-network level conservation planning towards the species.

**Keywords:** Agriculture; Metapopulation; Occupancy/detection models; Imperfect detection, Presence signs

## 2.1. Introduction

Understanding the effects of land-use change on spatially structured populations has become a main goal in both wildlife ecology and conservation (Stoate et al., 2001; Tscharntke et al., 2005; Bennett et al., 2006; Henle et al., 2008). In this context, the metapopulation concept has provided a useful modeling framework by focusing mostly on the occupancy dynamics of habitat patches, while disregarding local population dynamics (Driscoll, 2007; Pita et al., 2013). This common feature to most patch-level occupancy models make them particularly attractive to estimate metapopulation spatial patterns and dynamics over time, both because they are analytically tractable and require simple presence-absence sampling schemes, which are relatively easy to plan and implement (Bailey et al., 2004; MacKenzie and Bailey, 2004). However, it is now widely acknowledged that accurate estimates of occupancy and the factors affecting it, requires due consideration on the possibility that species may be imperfectly detected during surveys, which increases the chances for false absences, lowering the strength of inferences that can be made, by rendering at best only naïve estimates of occupancy (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005). To deal with this problem, detection probability should be accounted for during the

modeling process, which requires repeating surveys in at least some of the habitat patches within a relative short time, during which occupancy status is assumed to be unchanged (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005). Although occupancy models explicitly incorporating imperfect detection have been routinely implemented to allow unbiased estimation of occupancy probability, and the covariates affecting it, there are still many examples of species for which detection probabilities have been recurrently ignored when modeling occupancy. This may have important implications in model results, which in turn may lead to biased inference, and misleading wildlife management and conservation (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005).

The southern water vole (*Arvicola sapidus*, Miller 1908 Rodentia, Cricetidae) is a small mammal restricted to the Iberian Peninsula and France, which is classified as 'Vulnerable' by the International Union for Conservation of Nature (IUCN) Red List (Rigaux et al., 2008). Populations of this species are declining in most parts of its range, mainly due to habitat loss and degradation resulting from human activities (Rigaux et al., 2008; Pita et al., 2010; Mate et al., 2013; Ventura, 2012). Suitable habitats for the southern water vole are mostly associated to stable banks of water courses, in muddy soil, dominated by herbaceous or shrub vegetation (Fedriani et al., 2002; Román, 2007; Román, 2010; Ventura, 2012; Mate et al., 2013). The species is largely dependent on grasses, sedges, and reeds, which are an important source of food, nesting site, and protection from predators (Román, 2007; Román, 2010; Ventura, 2012; Mate et al., 2013). Similarly to its congeneric *A. amphibius*, the southern water vole usually forms discrete and easily recognizable breeding colonies, which in heterogeneous and fragmented landscapes are often separated by hundreds of meters from each other, being connected only via dispersal movements (mean of about 600-800m, Román, 2007; Centeno-Cuadros et al., 2011). The species has thus been often seen as an ideal model to examine whether a metapopulation approach can yield useful insights for assessing its population spatial structure and dynamics (Fedriani et al., 2002; Centeno-Cuadros et al., 2011; Pita et al., 2013). However, to date, studies relying on occupancy profiles of southern water voles have been largely based on presence sign surveys, and under the assumption that the species is perfectly detected across space and time, even though it is likely that detection probability may vary with both site- and survey-specific variables (Fedriani et al., 2002; Román, 2003; Román, 2010; Mate et al., 2013)

Given the need to assess population trends for the threatened southern water vole, and the lack of studies modeling occupancy probabilities of the species accounting for imperfect detection, this study aimed at providing further insights on the factors shaping the occupancy patterns and dynamics of this species in Mediterranean farmland, while taking into account for eventual variation in detection probabilities relative to both site- and survey-specific covariates. Specifically, we used a spatially-explicit modelling approach to assess seasonal occupancy probabilities of southern water voles relative to patch area, isolation, presence of water, and vegetation composition and structure; while simultaneously testing for the potential effects of vegetation variables, sampling effort, observer experience, and rainfall on the detection probabilities of the species based on presence-sign searches. In line with the predictions from metapopulation theory, we expect that patch occupancy of southern water voles should be positively related to patch size, and negatively so with patch isolation (Hanski, 1998; Hanski, 1999; Fedriani et al., 2002; Hanski and Ovaskainen, 2003; Pita et al., 2013). In addition, according to habitat preferences of the species (Román, 2007; Pita et al., 2011), the probability of patch occupancy should increase with the presence of water, and the increasing of both vegetation cover and high. On the other hand we expect that detection probabilities may vary not only with local vegetation characteristics, but also with survey-related variables. In particular, detection probabilities should be higher for experienced observers, at increased sampling efforts, and by the end of the study, decreasing for instance with increasing rainfall, which may wipe off voles droppings (Román, 2003; Pita et al., 2013). Overall, results from the study are expected to provide further insights regarding the responses of southern water vole patchy populations to current land-uses in fragmented Mediterranean farmland; and to discuss the importance of eventual heterogeneous detection probabilities on the strength of inferences made, and its implications for large-scale (landscape- and regional-level) monitoring programs targeting the conservation of the species.

## **2.2. Material and Methods**

### **2.2.1. Study area and species**

The study was carried out on the coastal plateau of south-western Portugal (37° 21' - 38° 04' N, 08° 51' - 08° 30' W, see Fig. 1). Climate is Mediterranean with oceanic influence, and mean monthly temperature ranging between 6 and 29 °C (Pita et al., 2007). The average annual rainfall is around 650 mm, of which >80% falls between October and March (wet season). The study area is an

agricultural landscape of about 247.6 ha, characterized by different land covers dominated by pastures, fodder crops, and silage corn or sorghum (Pita et al., 2007). Wood cover is limited to a few woodlots and hedges with pines and eucalyptus delimiting irrigated fields, while natural cork oak woodlands, shrubs and marshy vegetation are most frequent in the surroundings of extensive agricultural fields (Pita et al., 2009). Surface waters are mostly associated with small intermittent streams and temporary ponds which frequently dry out in summer, whereas permanent water bodies are scarce and mostly associated with irrigation infrastructures (Pita et al., 2013). Over the past three decades, agricultural practices have strongly intensified, particularly through expansion of cultivated lands, associated to the frequent use of pesticides and chemical fertilizers, with detrimental impacts on biodiversity (Beja and Alcazar, 2003; Pita et al., 2007; Pita et al., 2009).

Water voles in the study area are restricted to disperse patches of tall and dense herbaceous vegetation dominated by grasses, sedges, rushes, and reeds, which often occur in soft bank-margins of ponds, small streams, and irrigation ditches (Pita et al., 2010, 2011, 2013)(see Fig. 1). Within habitat patches, water voles form discrete and easily recognized breeding colonies with individuals typically showing strong site fidelity to their home ranges. Mean lifespan of individuals is around 3–5 months, and mating may occur within polygynous or monogamous systems, depending on local habitat quality and population density (Pita et al., 2010, 2011, 2013). Seasonality in food availability (greater during the wet season) is considered as key factor influencing population dynamics and breeding patterns (Pita et al., 2010). In addition, there is evidence for local extinction and colonization events, suggesting that, similarly to the congeneric *A. amphibious* populations in many regions from Northern Europe (MacPherson and Bright, 2011), metapopulation dynamics may critically affect the likelihood of species persistence in agricultural mosaics (Pita et al., 2013).

### **2.2.2 Vole surveys and explanatory variables**

Surveys were carried out in two occasions, the first from November to December 2013, corresponding to the beginning of the wet season, and the second from February to March 2014, which is when species reproduction rates and dispersal events were presumably higher (Román, 2007; Pita et al., 2013). Suitable habitat patches for water voles were identified during systematic field surveys, and included all areas dominated by tall and wet herbaceous vegetation, independently of the current occupancy status by the species (Pita et al., 2007, 2011, 2013).

Habitat patches were mapped through GPS recordings made along their borders and information was later incorporated in a vector based Geographic Information System (GIS, QGIS 2.0.1, Dufour, 2013). The minimum area for patch identification was 30 m<sup>2</sup>, which is about the minimum individual core area size recorded for the species (Pita et al., 2010). Patches were considered as distinct units if they were separated by inhospitable habitat (the matrix) decreasing the likelihood of individual movements. This included narrow areas (e.g. about 2 m wide) that were considered to act as potential barriers to voles daily movements, such as roads, houses, and concrete walls delimiting farmland properties (Pita et al., 2013). From the SIG we extracted for each patch its size (PS) and the distance to the nearest patch (DNP) (see Table 1).

Water voles presence was recorded from field searches for their typical presence signs, including fresh latrines or scattered faeces, tunnels, burrows and grass clippings. These signs are easily recognizable in the field, and provide a reliable basis for large scale surveys of the species (Fedriani et al., 2002; Román, 2007; Mate et al., 2013; Pita et al., 2013). A double-observer approach was implemented to estimate observer bias associated to detection probability (MacKenzie et al., 2002; MacKenzie et al., 2003; Mackenzie and Royle, 2005). An experienced observer (EO, >1 year of regular surveys of southern water voles, based on presence signs), and a naïve observer (NO, ≈1 week of specific training before the beginning of the study) sampled each habitat patch simultaneously. Three time-based sampling efforts were alternately employed to estimate the potential effect of survey duration in detection probability. Specifically, water vole presence signs within habitat patches were searched using 15min/ha (SE1), 30min/ha (SE2), or 60min/ha (SE3). In addition, rainfall (RAIN) during vole surveys was also recorded (as a 0/1 binary variable) because precipitation is referred to negatively affect the detection and identification of water vole presence signs (Román, 2003).

Vegetation composition and structure were measured in each habitat patch using 5m-radius circular sampling plots (6 per ha), selected to cover the whole patch area as much as possible. In each sampling plot, the percentage of cover by herbs (HC) and shrubs (SC) was visually estimated using four classes of vegetation cover: 0-25%, 26-50%, 51-75%, and 75-100%. In addition, five points (one at the centre of the plot, and another 4 about five meters apart, at each cardinal direction) were used to record the higher interception of herbs (HH) and shrubs (SH) with a metallic rod, placed vertically at each point (Pita et al., 2006). Measurements regarding vegetation cover and high were later used to estimate mean values for each habitat patch. We further recorded the presence (0/1) of

water in each habitat patch (PW), as this variable may also affect water voles (e.g. Ventura, 2012; Mate et al., 2013, see Table1).

### **2.2.3 Data analysis**

Prior to statistical analysis, all variables except binary descriptors were transformed to approach normality and to reduce the influence of extreme values, using the logarithmic transformation for continuous variables and the arcsine transformation for percentage data (Zuur et al., 2010). Pairwise Pearson correlations between continuous independent variables describing habitat patches were then performed, in order to exclude highly correlated variables ( $>0.4$ ) (Zuur et al., 2010), and thus avoid collinearity problems when modelling the factors affecting water vole probability of detection ( $p$ ) and occupancy ( $Psi$ ).

Differences in habitat patch variables between sampling occasions were analyzed based on univariate linear mixed modelling (GLMM), using the package 'lme4' (Bates et al., 2014) for R 3.1.0 (R Development Core Team 2014). Two models were built for each variable: one including the season as fixed factor, and the patches as random effect (season model), and another including only the random effect (null model). Eventual differences between seasons were evaluated by comparing the relative support of the season model relative to the null model, based on the Akaike Information Criterion (AIC, Burnhan and Anderson, 2002).

We use a multi-season implicit dynamic occupancy modelling approach to analyse the factors influencing water vole detection ( $p$ ) and occupancy probabilities ( $Psi$ ), which consists in applying single-season spatially-explicit occupancy models separately for each season (MacKenzie et al., 2005; Hossack et al., 2013; Ellis et al., 2014). In contrast to explicit-dynamics approaches, implicit dynamic models are mostly focused on the spatial patterns of occupancy at each season, or the net change over time, and do not explicitly estimate local colonization and extinction probabilities. This modelling approach is also known as the 'random changes in occupancy' multi-season model (MacKenzie et al., 2005), as it assumes that the probability of a species not going locally extinct at a previously occupied patch is equal to the probability of colonization of a previously unoccupied patch (MacKenzie et al., 2005). We have refrained from building explicit dynamic occupancy models (e.g. integrated habitat-occupancy models, MacKenzie et al., 2011), because implicit dynamic models greatly facilitate model fitting when few extinction and colonization occur in the data (MacKenzie et al., 2005). Models were implemented in the program PRESENCE 6.4 (Hines 2006), which uses a maximum-likelihood

approach to estimate  $p$  and  $Psi$  as a function of covariates, using logit link functions, and assuming that (i) sites are closed to changes in site occupancy at the species level during each season, (ii) species are not detected if they are absent and may or may not be detected if they are present, and (iii) detection at one site is independent of detection at all other sites (MacKenzie et al., 2002; Urban and Swihart, 2009; Durso et al., 2011).

Different model specifications underlying different ecological hypothesis regarding the factors affecting  $Psi$  and  $p$  were considered. Important factors were identified through an AIC-based multi-model selection procedure designed to first assess the best model explaining  $p$ , and then model  $Psi$ , considering the best model found for  $p$  (Gooch et al., 2006; Kirlin et al., 2006; Duren et al., 2011). In each case, multivariate models were built and included in the multi-model selection procedure only when individual variables involved showed higher support than the model with no effects ([i.e. the null model  $Psi(.) p(.)$ ]). Specifically, models including independent variables potentially affecting  $p$  (i.e. sampling effort, observer experience, rainfall, patch vegetation characteristics, and time since the beginning of the study), while maintaining  $Psi$  constant, were first ranked according to their AIC, and the best model was found based on AIC differences and weights (Gooch et al., 2006; Kirlin et al., 2006; Symonds and Moussalli, 2011). Models including independent variables presumably affecting  $Psi$  (patch-specific variables describing their size, isolation, vegetation characteristics, and presence of water) were then ranked based on AIC differences and weights, while controlling for the factors likely to affect  $p$  (Duren et al., 2011; Durso et al., 2011).



**Table 1** – Summary statistics of each independent variable between sampling seasons

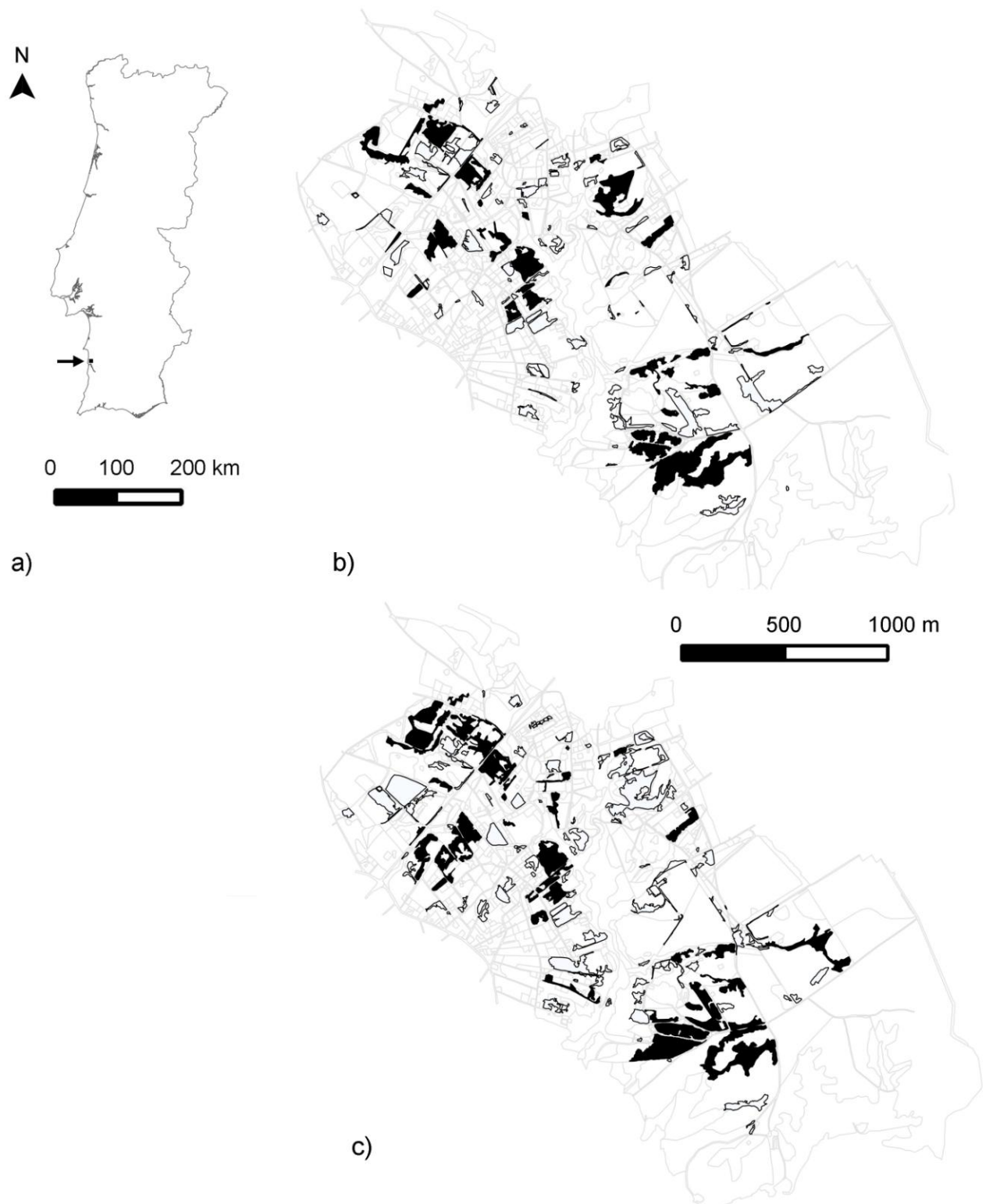
		<b>Session 1 (n=131)</b>		<b>Session 2 (n=138)</b>	
<b>Variable (units)</b>	<b>Code</b>	<b>Mean <math>\pm</math> se</b>	<b>Range</b>	<b>Mean <math>\pm</math> se</b>	<b>Range</b>
Patch size (m <sup>2</sup> )	PS	2755.959 $\pm$ 42.108	31.7-51841	3250.022 $\pm$ 40.400	50-33128
Distance to the nearest patch (m)	DNP	24.906 $\pm$ 0.246	2-202.233	13.341 $\pm$ 0.129	2-115.879
Herb cover (%)	HC	0.849 $\pm$ 0.001	0.44-0.88	0.870 $\pm$ 3E-04	0.63-0.88
Shrub cover (%)	SC	0.325 $\pm$ 0.002	0-0.88	0.276 $\pm$ 0.002	0-0.88
Herb height (m)	HH	45.067 $\pm$ 0.120	20.625-91.25	37.351 $\pm$ 0.077	18.75-80.625
Shrub height (m)	SH	81.505 $\pm$ 0.335	0-300	73.213 $\pm$ 0.435	0-400
Presence of water (0/1)	PW	0.267 $\pm$ 0.003	0-1	0.522 $\pm$ 2.633 E-05	0-1
Rain (0/1)	RAIN	0.191 $\pm$ 0.003	0-1	0.399 $\pm$ 0.004	0-1

## 2.3 Results

### 2.3.1. Overall patterns

A total of 131 and 138 habitats were identified and mapped in the first and second seasons respectively. Overall, the total habitat area increased from the first (36.35 ha) to the second season (45.51 ha). Twelve of the patches identified in the first season were joined to adjacent patches during the second season, resulting in a total of 108 and 96 identifiable patches 'common' to both seasons during the first and second season, respectively. From the first season to the second season 39 new habitat patches were identified and mapped and 23 patches have disappeared due to habitat conversion. Overall, water vole presence signs were found in 38 and 39 patches in the first and second seasons respectively (Fig. 1). Altogether there were 12 colonization events, of which 3 were into newly available patches, and 11 extinctions, of which 2 were deterministic (i.e. due to habitat conversion).

The GLMM analysis to assess seasonal effects in independent variables indicated that there was support for seasonal effects in the case of PS, DNP, HC, HH, PW, and RAIN. Specifically, PS, HC, PW, and RAIN increased from the first to the second season, while DNP and HH decreased. No season effects were found for variables describing the shrub layer (SC and SH). (See Tables 1 and 2)



**Fig. 1** – a) Location of the study area; b) suitable habitats identified and mapped during the first season; c) suitable habitats identified and mapped during the second season. White polygons correspond to empty patches, while black polygons correspond to occupied patches.

**Table 2** – GLMM AIC-based comparison of independent variables between seasons. Lines in bold indicate the cases in which there was higher support for the seasonal model (Delta AIC>2)

	<b>AIC Seasonal model</b>	<b>AIC 'null' model</b>	<b>Delta AIC</b>
<b>Patch Size (PS)</b>	<b>436,4</b>	<b>439,8</b>	<b>3,4</b>
<b>Distance to the Nearest Patch (DNP)</b>	<b>334,2</b>	<b>347,3</b>	<b>13,1</b>
<b>Herb Cover (HC)</b>	<b>-665,1</b>	<b>-657</b>	<b>8,1</b>
Shrub Cover (SC)	58,1	57,6	0,5
<b>Herb Hight (HH)</b>	<b>-341,1</b>	<b>-314</b>	<b>27,1</b>
Shrub Hight (SH)	451	452,2	1,2
<b>Presence of water (PW)</b>	<b>347,5</b>	<b>376,6</b>	<b>29,1</b>
<b>Rain (RAIN)</b>	<b>333,8</b>	<b>347,8</b>	<b>14</b>

### 2.3.2 Water vole detection and occupancy probabilities

Estimated detection probabilities of water voles presence-signs averaged ( $\pm$ se)  $0.71 \pm 0.07$  and  $0.81 \pm 0.05$  for the first and second seasons, respectively. None of the univariate models including the variables expected to affect  $p$ , while keeping  $\Psi$  constant, showed higher support than the null model [ $\Psi(\cdot) p(\cdot)$ ] (see Appendix Table A1). For instance, considering the survey-specific covariates tested, although the naïve observer apparently exhibited lower detectability than the experienced observer ( $0.66 \pm 0.08$  against  $0.78 \pm 0.08$  in the first season, and  $0.77 \pm 0.07$  against  $0.87 \pm 0.06$  in the second season), the differences were not significant. Likewise, there was no support for differences in detectability among the sampling efforts considered (estimates from the lowest to the highest sampling efforts of  $0.65 \pm 0.11$ ,  $0.79 \pm 0.10$ , and  $0.73 \pm 0.12$  for the first season; and  $0.79 \pm 0.09$ ,  $0.86 \pm 0.10$ , and  $0.82 \pm 0.08$  for the second season). Detection probability under rainfall tended to be higher than under no rainfall ( $0.86 \pm 0.10$  against  $0.67 \pm 0.08$  for the first season, and  $0.83 \pm 0.07$  against  $0.80 \pm 0.08$  for the second season), but again, the differences were not significant. Therefore, models to test the factors influencing  $\Psi$  where built considering  $p$  constant [ $p(\cdot)$ ].

Estimated probability of occupancy after controlling for imperfect detection was relatively constant between seasons, averaging ( $\pm$ se)  $0.31 \pm 0.05$  during the first season, and  $0.29 \pm 0.04$  in the second seasons (naïve estimates were  $0.29$  or  $0.28$ , respectively). Univariate models including variables expected to influence  $\Psi$ , indicated high support for the effects PS, DNP, and PW during both seasons (see

Appendix, TableA2), and so the set of candidate models considered included the 7 possible model combinations with this variables, plus the null model [ $Psi(.)$   $p(.)$ ]. AIC-based model ranking indicated that the best model explaining  $Psi$  during the first season included the 3 variables considered (Table 3). This model was also supported during the second season, although the model without the effect of DNP also received considerable support (Table3). These best supported models for each season indicated the  $Psi$  was in general positively affected by PS and PW and negatively affected by DNP. However, there seems to be a weaker importance of DNP during the second season (Tables 3 and 4), along with a stronger importance of PS and some weakening of PW (Table 4).

**Table 3** - AIC-based ranking of the 7 candidate models to assess the factors affecting  $Psi$  of water voles in each season considered.

	Modelo	AIC	Delta AIC	AIC weighed
<b>Session 1</b>	$Psi$ (PS,DNP,PW) $p(.)$	198,15	0.00	0,748
	$Psi$ (PS,PW) $p(.)$	200.59	2,44	0,2208
	$Psi$ (DNP,PW) $p(.)$	204,64	6,49	0,0291
	$Psi$ (PW) $p(.)$	210.04	11,89	0,002
	$Psi$ (PS,DNP) $p(.)$	219,63	21,48	0.0000
	$Psi$ (PS) $p(.)$	220.36	22,21	0.0000
	$Psi$ (DNP) $p(.)$	233,75	35,6	0.0000
	$Psi$ (.) $p(.)$	237.61	39,46	0.0000
<b>Session 2</b>	$Psi$ (PS,DNP,PW) $p(.)$	200,82	0.00	0,4195
	$Psi$ (PS,PW) $p(.)$	201.12	0,3	0,3611
	$Psi$ (PS,DNP) $p(.)$	203,27	2,45	0,1232
	$Psi$ (PS) $p(.)$	203.77	2,95	0,096
	$Psi$ (DNP,PW) $p(.)$	217,49	16,67	0,0001
	$Psi$ (PW) $p(.)$	221.16	20,34	0.0000
	$Psi$ (DNP) $p(.)$	228,34	27,52	0.0000
	$Psi$ (.) $p(.)$	233.11	32,29	0.0000

**Table 4** – Coefficients of the effects of the most supported models for each season

Variables	Estimates ( $\pm$ se) of coefficients		
	Best model session 1	Best models session 2	
	<i>Psi</i> (PS,DNP,PW) <i>p</i> (.)	<i>Psi</i> (PS,DNP,PW) <i>p</i> (.)	<i>Psi</i> (PS,PW) <i>p</i> (.)
Patch size	+ 1.223455	+ 1.774817	+ 1.887870
Distance to the nearest patch	- 1.274010	- 0.886353	—
Presence of water	+ 2.694650	+ 1.021381	+ 1.028317

## 2.4 Discussion

Knowledge of the factors influencing animal distribution and abundance at large (landscape/regional) spatial scales is critical in many areas of ecological research, management, and conservation (Hanski, 1998; Lindenmayer and Fischer, 2006; Bennett and Saunders, 2010; Frey et al., 2012;). Presence-absence data have become popular for monitoring populations at the landscape and regional scales, particularly for species that are discontinuously distributed as patchy populations or metapopulations (Opdam, 1991; Hanski, 1994; Moilanen and Hanski, 1998; Hanski and Ovaskainen, 2003; MacPherson and Bright, 2011). However, for categorical presence-absence data to be useful in monitoring programs, estimates of species-specific detection probabilities should be incorporated into analytical approaches (MacKenzie et al., 2002). This may be particularly important when common methods to sample populations (e.g. capture-recapture, distance sampling) are impractical for large areas or elusive species, and surveys are mostly based on presence signs searches likely to involve detection probabilities less than 1. Such seems to be the case of studies measuring occupancy profiles of the southern water voles over large spatial scales, which have been mostly based on species presence sign surveys ignoring the possibility of imperfect detection (Fedriani et al., 2002; Pita et al., 2013, Mate et al., 2013).

In this study, the first of its kind attempted for the southern water vole, we estimated occupancy probability of the species in a patchy heterogeneous Mediterranean farmland mosaic, based on presence sign surveys, and incorporating imperfect detection. Our approach enabled us to estimate both detection and occupancy probabilities of the species in the selected farmland area, as well as the local and survey-specific factors affecting these estimates. We thus believe that our

results may have strong implications for designing monitoring programs for southern water vole patchy populations based on presence sign searches over large spatial scales; as well as for reducing bias in patch-network level conservation planning towards the species.

In general, our results suggest that presence sign searches repeated over relatively short time intervals seem to provide a reliable survey measure for estimating occupancy patterns and dynamics of southern water vole populations, as it yielded relatively high detection probabilities, and low variance relative to both patch- and survey-specific variables. This positive result may be in part due to the obviousness of southern water voles presence signs, which were relatively easy to detected visually (Román, 2003; Fedriani et al., 2002; Román, 2010; Mate et al., 2013), particularly at the time we conducted the surveys (from November to March), which is when habitat patches are mostly inundated, and voles density is expectedly higher than for instance during summer periods, when local habitat quality is presumably reduced (Román, 2007; Pita et al., 2013). Despite relatively high, detection probabilities of the species based on presence sign searches within limited time intervals was less than 1, suggesting that determining southern water voles distribution may still require occupancy modelling accounting for false negatives, by using replicated sampling as approached here. Nevertheless, under some circumstances (e.g. a single observer surveying each patch only once), it may be far simpler and more effective to perform single total area searches (e.g. Pita et al., 2013) rather than surveying patches at least twice along limited time intervals or spatial replicates. Indeed, given the apparently high detectability of water vole presence signs, total area searches are expected to greatly minimize imperfect detection (Pita et al., 2013), which may eventually be neglected in occupancy modelling.

Although our results support our initial prediction that detection probability of southern water voles based on time-limited searches for presence signs is less than one, they do not support the predictions that detection probability should be affected by either vegetation characteristics within habitat patches, observer experience, sampling effort employed, or rainfall during surveys. Failure to detect eventual effects of vegetation composition and structure on southern water voles detectability may be related to the fact that our presence sign searches began always in vegetation areas that southern water voles are known to prefer (e.g. wettest parts of humidity gradients found within patches, e.g. Pita et al., 2011). This may also explain why the sampling efforts used here did not affect detection probabilities, which suggests that, although future survey efforts should attempt to standardized sampling, surveying the species with relatively low sampling efforts

will probably not sacrifice greatly the detectability of southern water voles in patchy environments. On the other hand, our results suggest that, although naive observers may have slightly lower detection probabilities than experienced observers, the differences may be neglected, as long as inexperienced observers are trained prior to the survey. Thus training may be an efficient way to reduce observer-specific heterogeneity in detecting southern water voles presence signs, and should be important for large-scale monitoring programmes towards the species, eventually involving amateur volunteers, and only a few experienced observers (e.g. Román, 2010). Moreover, the lack of support for a negative effect of rainfall during the surveys on detection probabilities, contradicts our initial prediction that rainfall may reduce the chances of finding for instance voles droppings (Román, 2003; Pita et al., 2013). In fact there was a tendency for higher detectability during rainy days, which though non-significant, may suggest that eventual wipe off of voles presence signs by rain may probably be compensated by the possible greater activity of voles during rainy periods, particularly during the breeding season (Pita et al., 2013). This may in part explain why detection probabilities were apparently higher during the second season, which was when precipitation, and habitat quality and quantity were also higher, and local population densities, reproduction, and breeding were probably greater (Román, 2007; Pita et al., 2013).

As for occupancy of southern water voles in our study area, our spatially explicit patch occupancy modelling approach showed that if we had assumed that species was absent from sampled habitat patches at which presence signs were not found, we would naively have estimated that 29% and 28% of patches were occupied in the first and second seasons, respectively. After accounting for imperfect detection, mean estimates of occupancy increased to 31% and 29% respectively. Although underestimates of this magnitude should not be critical, we stress that the assumption of perfect detection may still be unwarranted for the southern water vole, and should be accounted for in studies regarding occupancy patterns and dynamics of the species based on presence sign searches within limited time-intervals. In addition, results from this study also show that occupancy of southern water voles in Mediterranean farmland was not constant across space, increasing for instance with increasing habitat patch size and decreasing isolation. These results are consistent with the predictions from metapopulation theory (Hanski and Kuussaari, 1995; Hanski et al., 1996), and are in agreement with other studies relying on southern water vole occupancy profiles that assumed perfect detection (Fedriani et al., 2002; Mate et al., 2013; Pita et al., 2013). Furthermore, according to habitat preferences of the species at fine spatial scales (Fedriani et al.,

2002; Román, 2007; Román, 2010; Ventura, 2012; Mate et al., 2013), the presence of water within habitat patches increased the likelihood of occupancy by southern water voles. Thus, although southern water voles may cope well with seasonal droughts typical of Mediterranean landscapes (Fedriani et al., 2002; Pita et al., 2010; 2013), the species is more likely to persist in patches where water is available, as referred for the species in other systems (Mate et al., 2013). On the other hand, the lack of effects of local vegetation composition and structure on voles occupancy may be related to the fact that suitable patches were identified according to the vegetation preferences of the species, and thus eventual differences among patches probably were not a limiting factor shaping the species occupancy.

Although the effects of patch size, isolation, and presence of water on occupancy estimates of southern water voles in our study area were similar in both seasons considered, the effects of isolation received comparatively less support during the second season, which was when the distances among habitat patches significantly decreased, while habitat patch area increased. On the other hand, the presence of water within patches seemed to have slightly higher weight in the first sampling season, which can be related to the significantly reduced local availability of water relative to that estimated in second sampling season. Despite these differences, occupancy estimates were quite similar in both seasons, probably resulting from the balance between colonization and extinction events, and between patch appearance and disappearance episodes. Although we recorded only a small number of colonization and extinctions events (naive estimates of about 8.6% and 7.9%, respectively), results suggest that metapopulation dynamics of southern water voles should be an important process to be investigated in further detail, possibly involving long-term sampling of populations over multiple seasons. This would allow developing reliable explicit dynamic, integrated habitat-occupancy models (Mackenzie et al., 2011), which hopefully may also incorporate changes in local-level covariates, besides patch appearance-disappearance (e.g. seasonal changes in patch size, isolation, or internal quality), as well as information on local demography and dispersal either from life-trapping (e.g. Sutherland et al., 2012; in press), or non-invasive genetic sampling (e.g. Waits and Paetkau, 2005). Replicating survey efforts for a longer time would provide information on the population trends of southern water vole populations and help land managers to implement management plans benefiting the species. In addition, incorporating changes beyond habitat patches (e.g. in the matrix) should also provide further insights on the mechanisms driving occupancy patterns and dynamics of southern water voles in heterogeneous fragmented landscapes (Centeno-Cuadros et al.,



2001; Pita et al., 2013). Finally, research in other parts of the geographic range of the southern water vole is necessary to determine the true conservation status of this species and how it is responding to ongoing modification of its suitable habitat.

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### 3. Synthesis of main finding and implications

Agricultural intensification has become a threat for many species previously adapted to the traditional land-uses (Stoate et al., 2001; Henle et al., 2008; Stoate et al., 2009). In this context, understanding how changes in land-use may affect spatially structured populations has become a main goal, though also one of the most challenging to achieve, in both applied ecology and conservation (Stoate et al., 2001; Tschardt et al., 2005; Bennett et al., 2006; Henle et al., 2008). The metapopulation concept has been widely shown to provide a practical modeling framework to assess species responses to land-use change, by focusing mostly in the occupancy patterns and dynamics of habitats patches, and often disregarding local population dynamics (Driscoll, 2007; Pita et al., 2013). This common feature to most patch-level occupancy models make them particularly interesting to estimate metapopulation persistence over time, since they are analytically tractable and require simple presence-absence sampling schemes, which in turn are relatively easy to plan and implement (Bailey et al., 2004; MacKenzie and Bailey, 2004). However, although species presence/absence surveys are commonly used in occupancy modeling of spatially structured populations (Opdam, 1991; Hanski, 1994; Moilanen and Hanski, 1998; Hanski and Ovaskainen, 2003; MacPherson and Bright, 2011), predicting species presence requires consideration of detection probability of individuals (MacKenzie et al., 2002). Despite widely acknowledged, imperfect detection is still often disregarded in many studies aiming to estimate species occupancy patterns and dynamics (Fedriani et al., 2002; Pita et al., 2013; Mate et al., 2013). Accounting for imperfect detection during the modeling process requires repeated surveys in at least some of the habitat patches within a relative short time, during which occupancy status is assumed to be unchanged (MacKenzie et al., 2002; MacKenzie et al., 2003; Bailey et al., 2004; MacKenzie and Bailey, 2004; Mackenzie, 2005; Mackenzie and Royle, 2005).

This thesis addressed these issues, using the southern water vole (*Arvicola sapidus*) in south-west Portugal farmland as a case study. The southern water vole was considered a particularly interesting model, not only because populations in heterogeneous landscapes often spatially structured and show metapopulation-like dynamics, but also because of its "Vulnerable" conservation status and the urgent need for general information on population trends under landscape change. Although some studies based on presence-sing surveys have been made to assess the factors affecting occupancy patterns of southern water vole patchy populations or metapopulations in different systems (e.g. Fedriani et al., 2002; Mate et al., 2013; Pita et al., 2013;), the present study contributed as a first attempt to model

the occupancy probability of the species, while accounting for imperfect detection. To do so, we estimated occupancy probability of the species in a patchy heterogeneous Mediterranean farmland mosaic, based on repeated presence-sign searches conducted during two seasons (November-December and February-March), and incorporating imperfect detection. Specifically, we used a multi-season implicit dynamic occupancy modeling approach to analyze the factors influencing water vole detection and occupancy probabilities, which consisted in applying single-season spatially-explicit occupancy models separately for each season (MacKenzie et al., 2005; Hossack et al., 2013; Ellis et al., 2014). Factors expected to affect seasonal occupancy probabilities of southern water voles, included independent variables like patch area, isolation, presence of water, and vegetation composition and structure within surveyed patches; while those expected to affect detection probability included vegetation variables, sampling effort, observer experience, and rainfall during surveys.

This work showed that the detection probability of southern water voles in patchy Mediterranean farmland were relatively high ( $\approx 0.71$  and  $0.81$  for the first and second season, respectively), though no support was found for none of the variables expected to affect detection probabilities. These results suggest that surveying the species in patchy environments using relatively low sampling efforts will probably not sacrifice greatly its detectability. However, despite relatively high, detection probabilities of the species based on presence sign searches within limited time intervals was indeed less than 1, suggesting that assessing southern water voles distribution may still require occupancy modeling accounting for false negatives, by using replicated sampling as approached here. Although the study failed to detect any of the effects initially expected to affect the species detectability, it is recommended that future survey efforts should attempt to standardize sampling as much as possible. For instance, in relation to the observer experience, it should be highlighted that training volunteers before the surveys may be an efficient way to reduce observer-specific heterogeneity in detecting southern water voles presence signs, and should be particularly important for large-scale monitoring programmes towards the species, eventually involving amateur volunteers, and only a few experienced observers.

This study also found that after controlling for imperfect detection, occupancy of southern water voles in Mediterranean farmland was relatively low ( $\approx 29-31\%$ ) and spatially heterogeneous, increasing for instance with increasing habitat patch area and water availability and decreasing with isolation. Occupancy estimates were however quite similar across the two seasons considered, probably resulting from the balance between colonization and extinction events, and between patch



appearance and disappearance episodes. These results suggest that, metapopulation dynamics of southern water voles should be an important process to be investigated in further detail, possibly involving long-term sampling of populations over multiple seasons. Replicating survey efforts for a longer time would provide information on the population trends of southern water vole populations and help land managers to implement management plans benefiting the species. Also, incorporating changes beyond habitat patches (e.g. in the matrix) should also provide further insights on the mechanisms driving occupancy patterns and dynamics of southern water voles in heterogeneous fragmented landscapes (Centeno-Cuadros et al., 2011; Pita et al., 2013).

In the face of the current conservation needs of the species, and the general lack of knowledge regarding population responses to landscape change, this study provided evidence that designing monitoring programs for southern water vole patchy populations at landscape and regional scales, based on replicated presence sign surveys within limited time intervals, may be a reliable approach to estimate voles occupancy patterns and dynamics. This approach would help to improve patch-network level conservation planning towards the southern water vole in different parts of its geographic range, and thus to determine the true conservation status of the species, and how it is responding to ongoing modification of its suitable habitat.

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## Appendix 1

**Table A1** - PRESENCE ranked models for detection probability (p) in each season, showing that there are no independent variables with significant effects on detection probabilities.

<b>Model</b> (Season 1)	<b>AIC</b>	<b>deltaAIC*</b>	<b>Model</b> (Season 2)	<b>AIC</b>	<b>deltaAIC*</b>
<i>Psi</i> (.), <i>p</i> (SC)	236,12	1,49	<i>Psi</i> (.), <i>p</i> (NO)	233,75	-0,64
<i>Psi</i> (.), <i>p</i> (RAIN)	237,81	-0,2	<i>Psi</i> (.), <i>p</i> (HH)	234,31	-1,2
<i>Psi</i> (.), <i>p</i> (HH)	238,01	-0,4	<i>Psi</i> (.), <i>p</i> (HC)	234,94	-1,83
<i>Psi</i> (.), <i>p</i> (NO)	238,11	-0,5	<i>Psi</i> (.), <i>p</i> (RAIN)	234,99	-1,88
<i>Psi</i> (.), <i>p</i> (HC)	239,22	-1,61	<i>Psi</i> (.), <i>p</i> (SC)	235,11	-2
<i>Psi</i> (.), <i>p</i> (EFFORT)	240,5	-2,89	<i>Psi</i> (.), <i>p</i> (EFFORT)	236,85	-3,74
<i>Psi</i> (.), <i>p</i> (DAY)	238,11	-0,5	<i>Psi</i> (.), <i>p</i> (DAY)	234,78	-1,67
<b>1 group, Constant P</b>	<b>237,61</b>		<b>1 group, Constant P</b>	<b>233,11</b>	
*AIC (null model) - AIC (tested model)			*AIC (null model) - AIC (tested model)		

**Table A2** - PRESENCE ranked models for occupancy probability (Psi) in each season, showing the support for several tested variables, such as patch size, presence of water and distance to the nearest patch.

<b>Modelo</b> (Season 1)	<b>AIC</b>	<b>deltaAIC*</b>	<b>Modelo</b> (Season 2)	<b>AIC</b>	<b>deltaAIC*</b>
<i>Psi</i> (PW), <i>p</i> (.)	210,04	27,57	<i>Psi</i> (PS), <i>p</i> (.)	203,77	29,34
<i>Psi</i> (PS), <i>p</i> (.)	220,36	17,25	<i>Psi</i> (PW), <i>p</i> (.)	221,16	11,95
<i>Psi</i> (DNP), <i>p</i> (.)	233,75	3,86	<i>Psi</i> (DNP), <i>p</i> (.)	228,34	4,77
<i>Psi</i> (SC), <i>p</i> (.)	236,64	0,97	<i>Psi</i> (HH), <i>p</i> (.)	232,5	0,61
<i>Psi</i> (HH), <i>p</i> (.)	239,3	-1,69	<i>Psi</i> (SC), <i>p</i> (.)	232,65	0,46
<i>Psi</i> (HC), <i>p</i> (.)	239,5	-1,89	<i>Psi</i> (HC), <i>p</i> (.)	235,11	-2
<b>1 group, Constant P</b>	<b>237,61</b>		<b>1 group, Constant P</b>	<b>233,11</b>	
*AIC (null model) - AIC (tested model)			*AIC (null model) - AIC (tested model)		

Field data sheets from the "NETPERSIST" project, used to record southern water voles detection/non detection, and habitat-specific variables.

[illegible]

## CARACTERIZAÇÃO DE HABITATS

[illegible]